IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of

Applicants	: Kurt Friedrich Brandstadt et al.
Serial No.	: 10/791,951
Filed	: March 03, 2004
Title	: **METHODS FOR FORMING STRUCTURALLY DEFINED ORGANIC MOLECULES**
Docket No.	: DOG 0084 PA / 35319.50
Examiner	: R. Prouty
Art Unit	: 1652
Confirmation No.	: 1997

MAIL STOP RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

EFS Web Electronic Submission
October 11, 2007

Dear Sir:

DECLARATION UNDER 37 C.F.R. 1.132

Kurt F. Brandstadt, Thomas H. Lane, John C. Saam, and Joseph C. McAuliffe each declare that:

1. We are co-inventors of and are familiar with the present application Serial No.10/791,951.

2. In support of our application, we are submitting the additional information contained in the pages attached to our declaration. This additional information is taken from Examples 14-16 in our commonly-assigned PCT International Patent Application No. WO 2005/085459, which claims priority from this U.S. application Serial No. 10/791,951. The additional Examples demonstrate the ability of cutinase to catalyze the condensation of trimethylsilanol (Ex. 14), the ability of cutinase to catalyze the hydrolysis and condensation of dimethyldimethoxysilane (Ex. 15), and the ability of trypsin to catalyze the hydrolysis and condensation of diethyldiethoxygermane (Ex. 16). The processes described in these examples follows the teachings in our Serial No. 10/791,951.

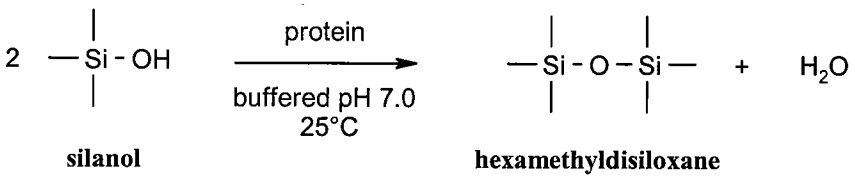
3. We further declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Kurt F. Brandstadt	Date
Thomas H. Lane	Date
John C. Saam	Date
Joseph C. McAuliffe	Date

Cutinase-catalyzed condensation of trimethylsilanol

A model study was performed in which a mono-functional silane was chosen to focus on the formation of molecules with a single siloxane bond during the *in vitro* condensation of trimethylsilanol (Scheme 6). The biocatalyzed reactions were formulated with a 5:1 trimethylsilanol to protein weight ratio (i.e. ~1,300:1 trimethylsilanol to cutinase mole ratio, 0.3 μmol cutinase) in 50 mM Tris-HCl buffered Milli-Q water (pH 7.0) at an ~10:1 solvent to monomer weight ratio. The closed (screw capped) two-phase reactions were conducted in inert glass vials at 25°C with magnetic stirring for 14 hours. Specifically, the reactions were conducted in silylated glassware. Since a silanol-functional glass surface could react with trimethylsilanol, the silylated glassware was necessary to create an inert glass surface. Prior to analysis, the aqueous reactions were extracted with THF in the presence of NaCl and filtered through a Whatman Autovial® 5 0.45 μm Teflon® filter. The reaction products were quantitatively analyzed by gas chromatography-flame ionization detection (GC-FID).

In this study, control reactions were defined as non-enzymatic reactions. Experiments conducted in the absence of a protein were defined as negative control reactions. Proteinaceous molecules such as bovine serum albumin (BSA) and porcine-γ-globulins (globulins) were used to study non-specific protein catalysis. Substantial condensation of trimethylsilanol was not observed in the negative control and non-specific protein reactions in comparison to the raw material (trimethylsilanol, Me_3SiOH). In review, cutinase catalyzed the condensation of trimethylsilanol during the formation of hexamethyldisiloxane (HMDS) under mild conditions. Although the condensation reaction was conducted in water, the enzyme-catalyzed reaction was promoted by the phase separation of the product. The immiscibility of the product, hexamethyldisiloxane, changed the equilibrium and promoted the condensation reaction in the presence of water. Since the aqueous medium was saturated with trimethylsilanol (i.e. a two-phase reaction mixture), the reactant would continue to enter the aqueous phase due to the dynamic equilibrium of the condensation reaction.

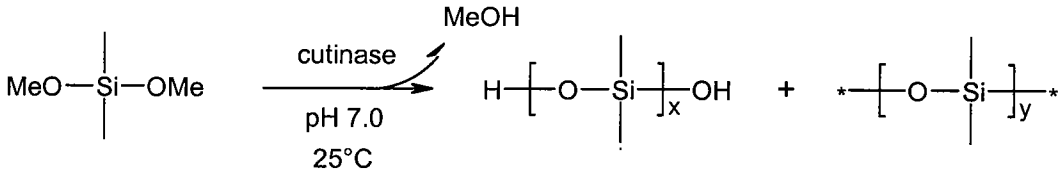


Scheme 6: Biocatalyzed condensation of trimethylsilanol.

Cutinase-Catalyzed Hydrolysis and Condensation of Dimethyldimethoxysilane

Dimethyldimethoxysilane (DMDM) was chosen as a model substrate to investigate the ability of cutinase to catalyze the *in vitro* hydrolysis and condensation of a multi-functional alkoxysilane under mild conditions (Scheme 7). The mild reaction conditions (i.e. low temperature, neutral pH) minimize chemically catalyzed condensation due to the increased concentration of silanol in the reaction mixture. The biocatalyzed reactions were initially formulated with an ~10:1 alkoxysilane to cutinase weight ratio (i.e. ~1,800:1 DMDM to cutinase mole ratio, ~5 μmol cutinase) in 50 mM Tris-HCl buffered Milli-Q water (pH 7.0) at an ~60% volumetric efficiency. Volumetric efficiency was defined as the weight % monomer measured as a percentage of the total weight of the liquids in the reaction (i.e. DMDM + water). The closed (screw capped) two-phase reactions were conducted in inert glass vials at 25°C with magnetic stirring for 24 hours. Specifically, the reactions were conducted in silylated glassware. Since a silanol-functional glass surface could react with the alkoxysilane in the study, the silylated glassware was necessary to create an inert glass surface. Prior to analysis, the aqueous reactions were extracted with THF in the presence of NaCl and filtered through a Whatman Autovial® 5 0.45 μm Teflon® filter. The reaction products were analyzed by GC-FID in a qualitative manner (i.e. area percent, Table 6).

In review (Table 6), the degree of polycondensation catalyzed by ~5 μmol of cutinase was not significantly greater than the negative control reaction conducted in the absence of cutinase over a 24-hour period. Consequently, the cutinase-catalyzed polycondensation reactions were replicated with an increased amount of enzyme (i.e. ~500:1 DMDM to cutinase mole ratio, ~20 μmol cutinase) over a longer period of time (5 days). Based on the degree of polycondensation obtained over an extended period of time (5 days vs. 24 hours) at two concentrations of enzyme (20 μmol vs. 5 μmol), cutinase was observed to catalyze the hydrolysis and condensation of DMDM (Table 7). Although the condensation reactions were conducted in water, the enzyme-catalyzed reactions were promoted by the phase separation of the products. Based on the estimated solubility of DMDM in water (~32 mg/mL), the concentration of DMDM (~1500 mg/mL) saturated the aqueous medium and created a two-phase reaction mixture. As the chain length of the linear siloxane molecules increase or cyclic siloxanes are formed, these molecules phase separate into the organic phase.



alkoxysilane **linear siloxane** **cyclic siloxane**

Scheme 7: Cutinase-catalyzed hydrolysis and condensation of dimethyldimethoxysilane.

Table 6: Cutinase-catalyzed hydrolysis and condensation of dimethyldimethoxysilane at 25°C after 24 hours.

		Area Percent[1]	
Siloxane[2]	$Me_2Si(OMe)_2$	Negative Control	Cutinase (~5 μmol)
L_1, ZO-$(Me_2SiO)_1$-Z	99.6 %	59.7 %	43.2 %
L_2, ZO-$(Me_2SiO)_2$-Z	0.4 %	32.9 %	43.3 %
L_3, ZO-$(Me_2SiO)_3$-Z	not observed	6.0 %	12.4 %
L_4, ZO-$(Me_2SiO)_4$-Z	not observed	0.9 %	0.6 %
L_5, ZO-$(Me_2SiO)_5$-Z	not observed	0.2 %	0.3 %
L_6, ZO-$(Me_2SiO)_6$-Z	not observed	0.1 %	not observed
D_3, $[Me_2SiO]_3$	not observed	0.2 %	0.2 %
D_4, $[Me_2SiO]_4$	not observed	0.1 %	not observed

1 The normalized area percent values were calculated from qualitative chromatographic data (GC).
2 L_x = dimethyl linear siloxane x = ZO-$(Me_2SiO)_x$-Z where x = 1-6 and Z = H &/or Me, Me = CH_3.
D_y = dimethyl cyclic siloxane y = $[Me_2SiO]_y$ where y = 3-4, Me = CH_3.

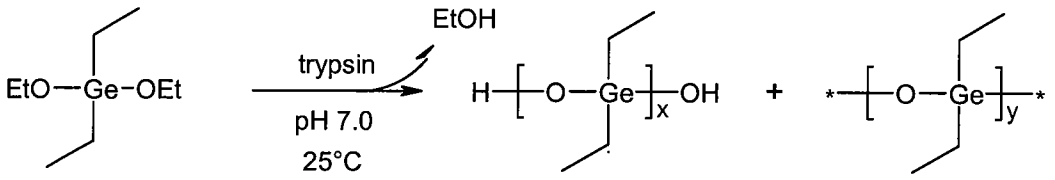
Table 7: Cutinase-catalyzed hydrolysis and condensation of dimethyldimethoxysilane at 25°C after 5 days.

	Area Percent[1]		
Siloxane[2]	Negative Control	Cutinase (~5 μmol)	Cutinase (~20 μmol)
L_1, ZO-$(Me_2SiO)_1$-Z	27.2 %	6.5 %	0.7 %
L_2, ZO-$(Me_2SiO)_2$-Z	44.5 %	48.1 %	21.4 %
L_3, ZO-$(Me_2SiO)_3$-Z	22.3 %	34.3 %	38.5 %
L_4, ZO-$(Me_2SiO)_4$-Z	3.8 %	8.5 %	25.9 %
L_5, ZO-$(Me_2SiO)_5$-Z	0.8 %	1.3 %	7.9 %
L_6, ZO-$(Me_2SiO)_6$-Z	0.2 %	0.2 %	2.2 %
L_7, ZO-$(Me_2SiO)_7$-Z	0.1 %	not observed	0.5 %
D_3, $[Me_2SiO]_3$	0.9 %	0.9 %	2.2 %
D_4, $[Me_2SiO]_4$	0.2 %	0.2 %	0.7 %

1 The normalized area percent values were calculated from qualitative chromatographic data (GC).
2 L_x = dimethyl linear siloxane x = ZO-$(Me_2SiO)_x$-Z where x = 1-7 and Z = H &/or Me, Me = CH_3.
D_y = dimethyl cyclic siloxane y = $[Me_2SiO]_y$ where y = 3-4, Me = CH_3.

Trypsin-Catalyzed Hydrolysis and Condensation of Diethyldiethoxygermane

Diethyldiethoxygermane was chosen as an alternate substrate in order to investigate the ability of bovine pancreatic trypsin to catalyze the *in vitro* hydrolysis and condensation of an alkoxy-functional germanium molecule under mild conditions (Scheme 8). The mild reaction conditions (i.e. low temperature, neutral pH) minimize chemically catalyzed condensation due to the increased concentration of hydroxy groups in the reaction mixture. The reactions were formulated with an ~5:1 monomer to enzyme weight ratio (i.e. ~500:1 diethyldiethoxygermane to trypsin mole ratio, ~1 μmol trypsin) in 50 mM Tris-HCl buffered Milli-Q water (pH 7.0) at an ~5:1 solvent to monomer weight ratio. The closed (screw capped) two-phase reactions were conducted in inert glass vials at 25°C with magnetic stirring for 24 hours. Specifically, the reactions were conducted in silylated glassware. Since a silanol-functional glass surface could react with the monomer, the silylated glassware was necessary to create an inert glass surface. Prior to analysis, the aqueous reactions were extracted with THF in the presence of NaCl and filtered through a Whatman Autovial® 5 0.45 μm Teflon® filter. The reaction products were analyzed by gas chromatography-mass spectrometry (GC-MS) and electrospray ionization mass spectrometry (ESI MS). In comparison to a negative control reaction conducted in the absence of the enzyme, trypsin was observed to catalyze the hydrolysis and condensation of diethyldiethoxygermane during the preferential formation of hexaethylcyclotrigeroxane, $[Et_2GeO]_3$ where Et = CH_2CH_3.



Scheme 8: Trypsin-catalyzed hydrolysis and condensation of diethyldiethoxygermane